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(21) International Application Number: PCT/GB92/01291 (22) International Filing Date: 15 July 1992 (15.07.92) (30) Priority data: 9115245.4 16 July 1991 (16.07.91) GB (71) Applicant (for all designated States except US): IMPERIAL CHEMICAL INDUSTRIES PLC [GB/GB]; Imperial Chemical House, Millbank, London SW1P 3JF (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : LIEBERGESELL, Matthias [DE/DE]; Institut für Mikrobiologie, Georg-August-Universität, Grisebachstraße 8, D-3400 Göttingen (DE). STEINBÜCHEL, Alexander [DE/DE]; Institut für Mikrobiologie, Georg-August-Universität, Grisebachstraße 8, D-3400 Göttingen (DE).		(74) Agent: HUSKISSON, Frank, Mackie; Imperial Chemical Industries plc, ICI Group Patent Department, P.O. Box 6, Bessemer Road, Welwyn Garden City, Herts AL7 1HD (GB). (81) Designated States: AU, BB, BG, BR, CA, CS, FL, HU, JP, KP, KR, LK, MG, MN, MW, NO, PL, RO, RU, SD, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: PRODUCTION OF POLYALKANOATE (57) Abstract Genes encoding polyhydroxyalkanoate synthase, β -ketothiolase and acetoacetyl CoA reductase are isolated from the publicly available bacterium <i>Chromatium vinosum</i> . Recombinant genomes of plants or other species of bacteria which contain these genes are capable of producing polyalkanoate polymers. The nucleotide sequences of the said three genes have been determined.		

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PRODUCTION OF POLYALKANOATE

This invention relates to the production of polyhydroxyalkanoate by the culture of microorganisms which produce same.

5 Poly-3-hydroxybutyrate is a linear polyester of D(-)-3-hydroxybutyrate. It was first discovered in Bacillus megaterium in 1925. Polyhydroxybutyrate accumulates in intracellular granules of a wide variety of bacteria. The granules appear to be membrane bound and can be stained with Sudan
10 Black dye. The polymer is produced under conditions of nutrient limitation and acts as a reserve of carbon and energy. The molecular weight of the polyhydroxybutyrate varies from around 50,000 to greater than 1,000,000, depending on the
15 microorganisms involved, the conditions of growth, and the method employed for extraction of the polyhydroxybutyrate. Polyhydroxybutyrate is an ideal carbon reserve as it exists in the cell in a highly reduced state, (it is virtually insoluble),
20 and exerts negligible osmotic pressure.

Polyhydroxybutyrate and related poly-hydroxyalkanoates, such as poly-3-hydroxyvalerate and poly-3-hydroxyoctanoate, are biodegradable thermoplastics of considerable commercial importance.

25 The term "polyhydroxyalkanoate" as used hereinafter includes copolymers of polyhydroxybutyrate with other polyhydroxyalkanoates such as poly-3-hydroxyvalerate.

Polyhydroxyalkanoate is biodegradable and is broken down rapidly by soil microorganisms. It is thermoplastic (it melts at 180°C) and can readily be moulded into diverse forms using technology well-established for the other thermoplastics materials such as high-density polyethylene which melts at around the same temperature (190°C). The material is ideal for the production of biodegradable packaging which will degrade in landfill sites and sewage farms. The polymer is biocompatible, as well as biodegradable, and is well tolerated by the mammalian, including human, body, its degradation product, 3-hydroxybutyrate, is a normal mammalian metabolite. However, polyhydroxyalkanoate degrades only slowly in the body and its medical uses are limited to those applications where long term degradation is required.

Polyhydroxyalkanoate, produced by the microorganism Alcaligenes eutrophus, is manufactured, as a copolymer with of polyhydroxybutyrate and polhydroxyvalerate, by Imperial Chemical Industries PLC and sold under the Trade Mark BIOPOL. It is normally supplied in the form of pellets for thermoprocessing. However, polyhydroxyalkanoate is more expensive to manufacture by existing methods than, say, polyethylene. It is, therefore, desirable that new, more economic production of polyhydroxyalkanoate be provided.

An object of the present invention is to provide materials and a method for the efficient production of polyhydroxyalkanoate.

According to the present invention there is provided gene fragments isolated from the bacterium Chromatium vinosum and encoding PHA polymerase, acetoacetyl CoA reductase and β -ketothiolase.

5 Preferably the C.vinosum is of the strain designated D, available to the public from the Deutsche Sammlung für Mikroorganismen under the Accession Number 180.

10 The invention also provides a 16.5kb EcoRI fragment of C.vinosum DNA, designated PP10, hybridizable to a 5.2kb SmaI/EcoRI fragment, designated SE52 isolated from Alcaligenes eutrophus and known to contain all three of said genes responsible for expression of PHAs.

15 The invention further provides a fragment of the said PP10 fragment, designated SE45, encoding the PHA-synthase and β -ketothiolase genes and a region, designated SB24, encoding the acetoacetyl CoA reductase gene.

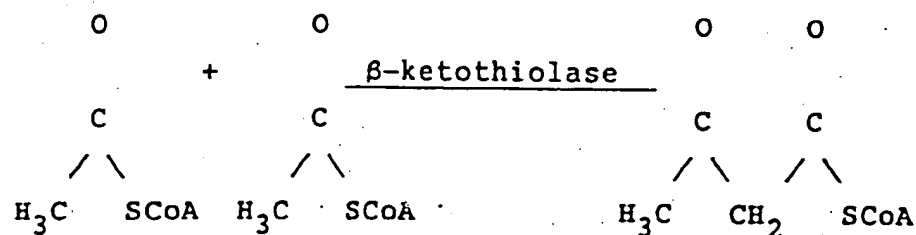
20 The invention also provides a recombinant genome of a microorganism, preferably a bacterium or a plant, which contains one or more of said fragments designated PP10, SE45 and region SB24.

25 Finally, the invention provides a method for the manufacture of PHAs, comprising culturing the microorganism Chromatium vinosum, or a bacterium of a different species having stably incorporated within its genome by transformation one or more PHA synthesising genes from Chromatium vinosum.

30 The biosynthesis of polyhydroxyalkanoate from the substrate, acetyl-CoA involves three enzyme-catalyse steps.

The three enzymes involved are β -ketothiolase, acetoactyl-CoA-reductase and polyhydroxy-

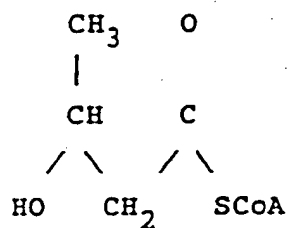
butyrate-synthase, the genes for which have been cloned from Chromatium vinosum. The three genes are known to facilitate production of polyhydroxyalkanoates, the reactions involved being represented as follows:.



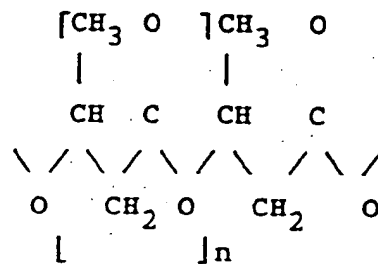
NADPH

NADP-linked
Acetoacetyl-CoA Reductase

NADP



Polyhydroxybutyrate-
synthase



The invention will now be described with reference to the accompanying drawings, of which;

Figure 1 is the physical map of the 16.5 kb EcoRI fragment of Chromatium vinosum DNA, designated PP10. The positions of the restriction sites and positions and names of the sub-fragments are shown. PHA-synthase and β -ketothiolase genes are located in fragment SE45 and acetoacetyl CoA reductase in region SB24;

Figure 2 is the map of PP10 showing the positions of the β -ketothiolase and acetoacetyl CoA reductase genes and of the PHA-synthase gene open reading frames ORF2 and ORF3.

Figure 3 is the complete nucleotide sequence of fragment SE45. The transcriptional start sites and terminators for the β -ketothiolase gene and for ORF3 and ORF 3 are shown. The positions of the "-10" and "-35" sequences are also shown, as are the positions of the putative ribosome binding sites ("s/d"). Translational start and stop (*) codon are also marked and the amino acid sequences of the β -ketothiolase, ORF2 and ORF3 are given.

Figure 4 shows the alignment of the amino acid sequences of Chromatium vinosum ORF3 with PHA polymerase of Alcaligenes eutrophus and PHA polymerases 1 and 2 of Pseudomonas oleovorans.

Figure 5 shows the complete nucleotide sequence of the DNA encoding PHA synthesis genes from Chromatium vinosum. The positions of PHA polymerase (phbC), acetoacetyl CoA reductase (phbB) and ketothiolase (phbA) genes are shown, and also the positions of ORF2, ORF4, ORF5 and ORF7.

Figure 6 shows the alignment of the amino acid sequences of ketothiolases encoded by C.vinsoum

(C.v.), A.eutrophus (A.e.), Zoogloea ramigera (Z.r.), Escherichia coli (E.c.), Saccharomyces uvarum (S.u.) and Rattus norvegicus (R.n.)

5 Figure 7 shows the alignment of the amino acid sequences of acetoacetyl CoA reductases encoded by C.vinosum (C.v.), A.eutrophus (A.e.) and Z.ramigera (Z.r.)

10 Figure 8 is a Table (Table 1) showing the heterologous expression in Escherichia coli of DNA fragments from C.vinosum. Activities of PHA biosynthetic enzymes expressed by the different fragments are shown. The levels of PHA accumulated in E.coli transformed with the fragments are also given.

15 EXAMPLE

The organism C.vinosum was a gift from Dr J. Imhoff of the University of Bonn, Germany.

1. Isolation of DNA fragments from C.vinosum encoding PHA synthesis genes

20 A 5.2 kb SmaI/EcoRI fragment (SE52), which codes for all three PHA biosynthetic genes has previously been isolated from Alcaligenes eutrophus [Schubert et al., J. Bacteriol. 170 (1988)]. This fragment was used to detect PHA biosynthetic genes of C.vinosum. EcoRI restricted genomic DNA of C.vinosum was blotted on to a nylon membrane and hybridized with biotinylated SE52 DNA. One signal appeared, representing a DNA fragment of 16.5 kb.

25 A λ L47 gene bank from C.vinosum genomic DNA was prepared and plates with approximately 800 plaques were blotted on to nylon membranes and hybridized with biotinylated SE52 DNA. One positive recombinant phage was isolated, which harboured a 16.5 kb EcoRI fragment, which was

30

designated PP10 (Figure 1). With PP10 and a 9.4 kb EcoRI/PstI subfragment (EP94) of PP10, the phenotype of the wild type could be restored in PHA-negative mutants of A.eutrophus.

5 Expression studies in E.coli (see below) showed that a 4.5 kb SmaI/EcoRI (SE45) subfragment of EP94 encodes for PHA synthase and β -ketothiolase. The nucleotide sequence of this fragment was determined by the dideoxy-chain
10 termination method of Sanger et al. with alkaline denatured double stranded plasmid DNA. The T7-polymerase sequencing kit of Pharmacia, Uppsala, Sweden, was used with 7-deazaguanosine-5'-tri-phosphate instead of dGTP. Most of the sequence
15 was determined with a set of unidirectional overlapping deletion clones generated by exonuclease III digestion. For sequencing regions which were not covered by the deletion plasmids synthetic oligonucleotides were used.

20 It was not possible to clone the 4.9 kb SmaI/PstI fragment PS49 in a multi-copy vector. Therefore, fragment EP94 (Figure 1) was treated with Exonuclease Bal31, ligated to Bluescript SK and transferred to E.coli Xl-1 Blue. A clone was
25 isolated which harboured Bluescript SK with a 5.5 kb fragment (B55) and which expressed β -ketothiolase and NADH-dependent reductase activity. 3146 base pairs of B55 were part of the SE45 fragment. The other part (approximately 2350
30 base pairs, SB24) has been sequenced applying the primer hopping strategy. The sequence and the position of the reductase gene on SB24 are known. The results of these studies, including the organisation of the PHA biosynthetic genes in

C.vinosum and the sites of the ketothiolase, reductase and PHA synthase genes are shown in Figure 2. The determination of the full sequence of SB24 is in progress.

5 2. Sequence analysis of the C.vinosum PHB Synthetic Genes

 The nucleotide sequence of SE45 is shown in Figure 3. The fragment size of SE45 is 4506 bp.

 2.1 PHB synthase

10 The fragment sequence corresponding to the PHB synthase gene is designated as ORF3. The determination of synthase activity of deleted plasmids containing SE45 (See below) gave evidence that expression of ORF2 is also required for
15 expression of synthase activity.

 ORF2 and ORF3 are transcribed as an operon. The determination of the transcription start site of ORF2 was conducted by S1 nuclease mapping. This site occurs at bp 3059 from the 3' end of SE45. A
20 putative "-10" site, given as 5'-ACAGAT-3' occurs at bp 3073-3078, and a "-35" site occurs at bp 3092-3099. A putative ribosome binding site occurs at bp 3040-3045. The translation start codon commences at bp 3030. The translation stop codon
25 occurs at bp 1958.

 The putative ribosome binding site of ORF3 occurs at bp 1907-1911. The translation start ATG for ORF3 occurs at bp 1899, and the translation stop codon at bp 833. Putative transcriptional
30 terminator sites occur at hairpin structures at bp 773-786 and 796-823.

 ORF2 encodes a polypeptide of 358 amino acids with a MW of 40525 da. ORF3 encodes a polypeptide of 356 amino acids with a MW of 39739 da. The gene

size of ORF3 is approximately 30% smaller as compared with the PHA polymerase genes of A.eutrophus and P. oleovorans. The alignments of the primary structures of C.vinosum PHA polymerase, A.eutrophus PHA polymerase and P.oleovorans PHA polymerases 1 and 2 are shown in Figure 4. Thus the ORF3 C.vinosum polymerase is shorter than the other polymerase enzymes, lacking the first 172 amino acids from the NH₂ terminus of the A.eutrophus PHA polymerase, and the first 148 amino acids of the Pseudomonas polymerases. The amino acid sequence of ORF3 exhibited an overall homology of 25% to the polymerase of A.eutrophus, with certain discrete regions of conserved sequence.

The amino acid sequence of ORF2 showed no significant homology to other enzymes in the NBRF gene bank.

2.2 β ketothiolase

The β ketothiolase and acetoacetyl CoA reductase genes are transcribed in opposite direction to ORF2 and ORF3 (Figure 2). A "-10" site in the identified ketothiolase promoter occurs at bp 3105-3111, and a "-35" site at bp 3082-3086. A putative ribosome binding site occurs at bp 3167-3171. The translation start signal occurs at bp 3181. The translation stop codon occurs at bp 4361.

The alignments of the primary structures of β ketothiolases from Chromatium vinosum and other sources are shown in Figure 5. Considerable homology is apparent between the amino acid sequences of ketothiolases from C.vinosum and other bacterial and non-bacterial sources.

2.3 Acetoacetyl CoA reductase

The alignments of the primary structures of acetoacetyl CoA reductases from C.vinosum, A.eutrophus and Z.ramigera are shown in Figure 6.

5 All three reductases are of similar chain length, while considerable homology is apparent between the sequences of reductases from these bacteria.

The Chromatium vinosum PHA synthetic genes therefore differ from the PHA synthetic genes of A.eutrophus and P.oleovorans in the following
10 respects:

i) Whereas A.eutrophus PHB polymerase, acetoacetyl CoA reductase and β ketothiolase genes are all transcribed as an operon, in C.vinosum the
15 ketothiolase and reductase genes are transcribed separately from the polymerase, and are transcribed in the opposite direction to the polymerase ORF3 and ORF2 genes.

ii) In contrast to A.eutrophus, where one
20 gene product is required for polymerase activity, in C.vinosum two gene products, represented by ORF2 and ORF3 are required for expression of polymerase activity.

iii) The C.vinosum ORF3 polymerase is 172
25 amino acids shorter, at the amino terminus, than the A.eutrophus polymerase, and 148 amino acids shorter than the P.oleovorans polymerases 1 and 2. The C.vinosum ORF3 shows only 25% homology with the primary sequence of the A.eutrophus polymerase.

30 iv) The A.eutrophus acetoacetyl CoA reductase enzyme involved in PHB synthesis is NADPH specific, while the C.vinosum enzyme exhibits a marked preference for NADH.

Between the structural genes for ketothiolase

and acetoacetyl CoA reductase of Chromatium
vinosum, two open reading frames (ORF4 and ORF5)
appear, and downstream from the reductase gene an
ORF7 has been identified (Figure 5). No additional
5 ORFs were identified in the PHA coding region of
A.eutrophus.

3. Expression of C.vinosum PHB synthetic genes in other bacteria.

With fragments PP10 and EP94 the ability to
10 synthesise PHB could be restored to PHB negative
mutants of A.eutrophus. Recombinant strains of the
PHB negative mutant A.eutrophus PHB-4, transformed
with these fragments, were able to synthesise
polymers containing 3-hydroxybutyrate and
15 3-hydroxyisovalerate at significant proportions,
when supplied with appropriate substrates.

Studies on expression of C.vinosum DNA
fragments in E.coli are presented in Table 1. Thus
E.coli transformed with plasmids containing
20 fragments PP10 and EP94 expressed PHB polymerase,
acetoacetyl CoA reductase and β ketothiolase
activities. They also synthesised PHB up to
between 10 and 12% dry weight. E.coli transformed
with plasmids containing fragment SE45 expressed
25 PHB polymerase and β ketothiolase, but not
acetoacetyl CoA reductase, and were unable to
synthesise PHB.

4. Polymer Biochemistry

The specific optical rotations of methyl
30 3-hydroxybutyric acid liberated by methanolysis of
PHB from C.vinosum (accumulated from acetate), from
A.eutrophus PHB-4 pHP1014::PP10 (accumulated from
fructose) and E.coli S17-1 pSUP202::PP10
(accumulated from glucose) were all negative. The

determined values of the specific optical rotation were similar to those for PHB isolated from A.eutrophus (accumulated from fructose).

CLAIMS

1. Gene fragments isolated from the bacterium Chromatium vinosum and encoding polyhydroxy-alkanoate (PHA) synthase, acetoacetyl CoA reductase and β -ketothiolase.
2. Gene fragments as claimed in claim 1 in which the Chromatium vinosum is of the strain designated D, available to the public from the Deutsche Sammlung für Mikroorganismen under the Accession Number 180.
5
3. A 16.5kb EcoRI fragment of Chromatium vinosum DNA, designated PP10, hybridizable to a 5.2kb SmaI/EcoRI fragment, designated SE52 isolated from Alcaligenes eutrophus and known to contain the genes encoding PHA-synthase acetoacetyl CoA reductase and β -ketothiolase.
5
4. A fragment of the PP10 fragment claimed in claim 3, designated SE45, encoding the PHA-synthase and β -ketothiolase genes.
5. A fragment of the PP10 fragment claimed in claim 3, designated SB24, encoding the acetoacetyl CoA reductase gene.

6. A recombinant genome which contains one or more of the fragments designated PP10, SE45 and region SB24 claimed in claims 3, 4 and 5 respectively.
7. A bacterium having incorporated in its genome one or more of the fragments designated PP10, SE45 and region SB24 claimed in claims 3, 4 and 5 respectively.
8. A plant having stably incorporated in its genome by transformation one or more of the fragments designated PP10, SE45 and region SB24 claimed in claims 3, 4 and 5 respectively.
9. A method for the manufacture of polyhydroxyalkanoates, comprising culturing the microorganism Chromatium vinosum, or a bacterium of a different species having stably incorporated within its genome by transformation one or more PHA synthesising genes from Chromatium vinosum.
10. A gene, encoding β -ketothiolase, having the nucleotide sequence shown in Figure 3.
11. A gene encoding polyhydroxyalkanoate synthase (phbC), having the nucleotide sequence shown in Figure 5.
12. A gene encoding acetoacetyl CoA reductase (phbB) having the nucleotide sequence shown in Figure 5.

FIG. 1

Physical map of the 16.5-kb *EcoRI* fragment PP10 and location of B55

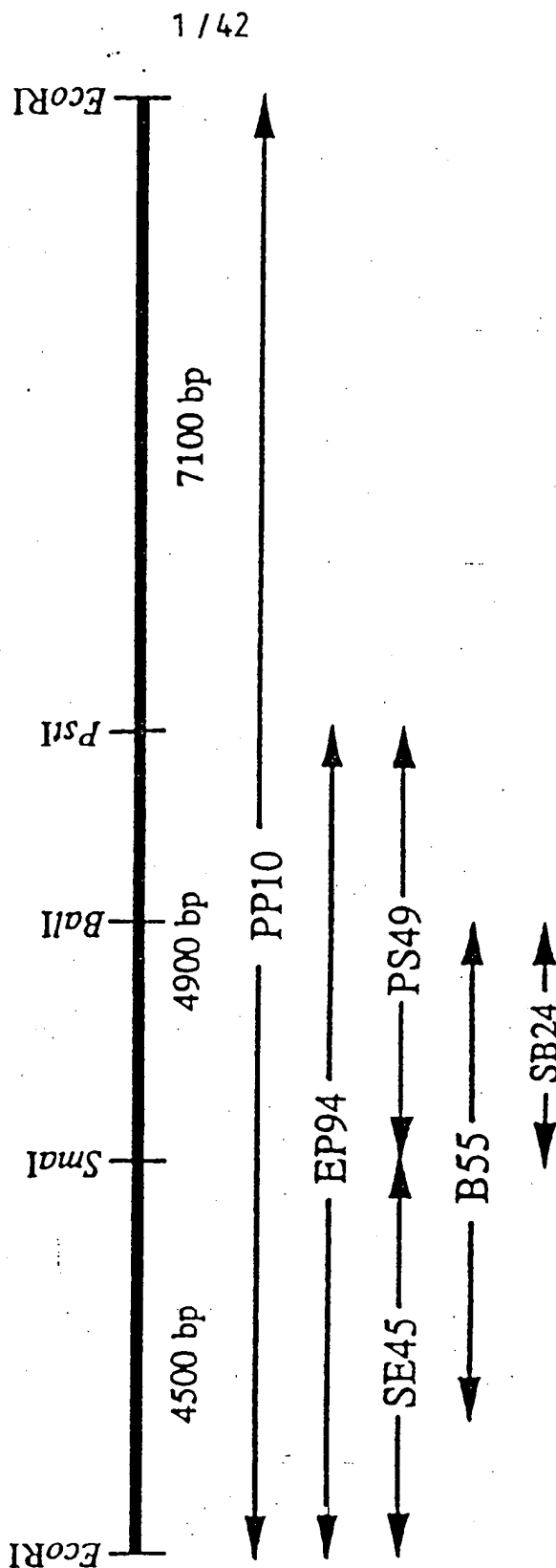
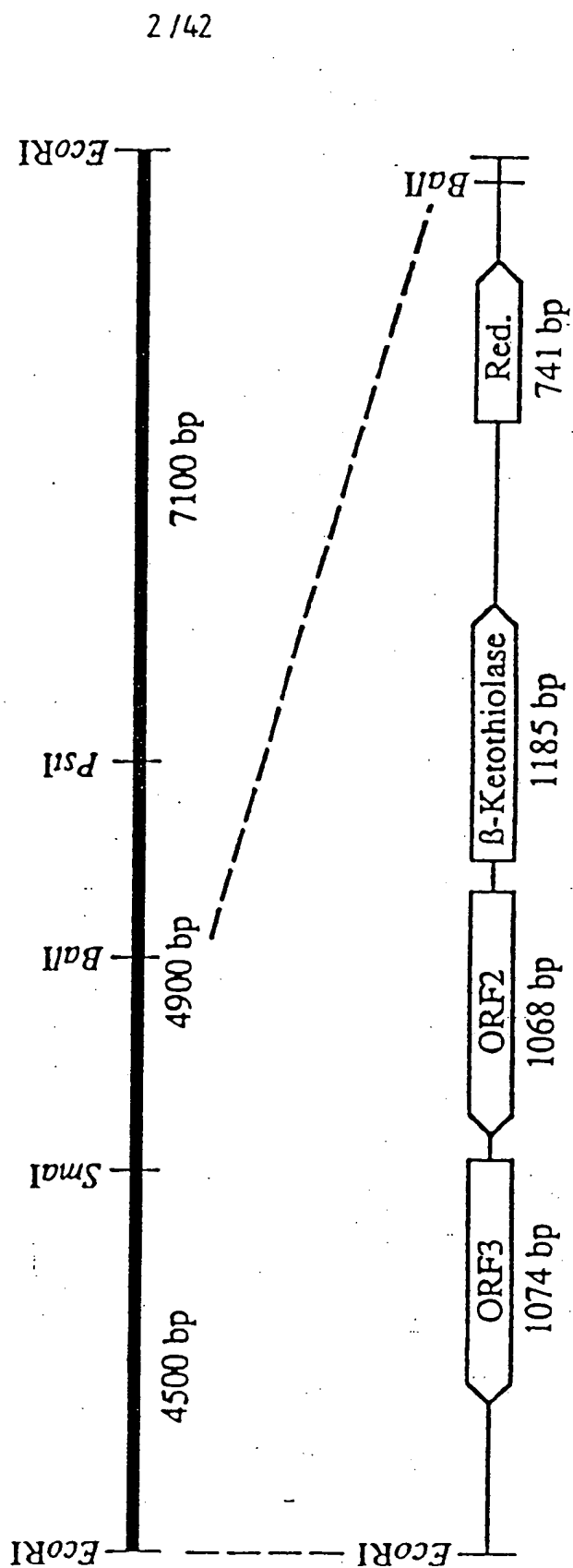


FIG. 2

Region of β -ketothiolase and reductase locus and relevant open reading frames of PHB-synthase



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FIG. 3 (1/9)

: Nucleotide sequence of fragment SE45

EcoRI

1 GCTTAAGTAGGTCCCGGTGATAGAGGTTGACGGCTCAAGCCTGAACTTG

101 CCGCGAAGTGTGCCCTGCCGACTAGGACTGAGGTAGGCTAGGCGTAGAG

201 TCACGAGGTAAGTACGACCTCTTAGGTGTCGGCCCCCGCCCGCTGGCTC


301 GGCAGGCCTAAGCGCTACTGCAGTCTCGCCCCGCAAAGAGCGTATAAGC

401 GGGTGGGGCTACACAGCCACAGCGCGCGGGGACAGCCCGACCGCGCGC

501 ACGCCGCTCTGTGCAGGCGGGACCGAGACCCTGACCTTTGTCGTTTCCTC

601 ACGGCTGCGGCTACAAGAAGCTCGTCGAGTAAGCCCAGTCACCCTAGCC

701 AATTGCACTTCCGGTCGAGGTTTCAGGCGAGCTACTCGCTGCTGCGGGCC

801  GCGGCCTTAACGAGGCCGCGAGCCCTTCGCAATAGCGAGCAACTCGGT
* R E N L W

901 CGGCTACACTGGCGGGCCCCTTCCGGTCGAGCCACATCAGGCCCGACGAC
G H I G G P F A L E T Y D P S S

1001 TCGCGCTTCTACAAGTTCGTGGCCCGTCCACTACAGGAAGTCCAGGTGAA
L A F I N L V P C T I D K L D V

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FIG. 3 (2/9)

AAGCTAAAGGTCTGGGACAGTGTACTTTGGGCTATCAGACGAGTACTCATG

CGCGGCCTACAGCTAAACAACCTAGCTTCCTGACTCGGCCGGACCTAAGGAC

GGAAACGCTGACCGCGGCCGAAGGACTCAGATGACTGGAAACGGCCGACGC

GTAGGGCGGTCAGTCAGTCGTAGGTTCCAACCTACCAACCGGCATTGTGCGC

GACCACCTTGTGACCCGGTGCGTAGGCGTACGGCCGCACGTTACAGGCCGG

CTAAGCCTGCGCGGAAGCACGTCTAGCTTCTCCAGAAAGGCCCACTCCGTC

GACCGCTGTAAAGATGTAGGTCCTAACTTAGTTCGGACCATATCCCCGCGG

ACTCAGGGCTCGCAGTAGCACCTACGGCAAGCTGCCGACTGCTGTGCTCG

GAATGGCTACCGGCCGCACTGAAGAAAGACGCGGAACGGCGACTGTATCTA

K G I A P T V E K Q A K G S V Y I

CAGTCAGGGAACTCGCGGGATCTCCGTAGGCCGCGTGGTCCACTAGGACC

T L G K L A R S A D P P V L H D Q

GGACAGGCGGGTCGTGGTGCGGCGGTAACCTCCTTCGGCAACAAGACCATCT

E Q G G L V V G G N L F G N N Q Y

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FIG. 3 (3/9)

1101 TCAGGAACTACTTGACTGCCTTCCAGAGCGGCCGGACTAGGCCCGACA
F D K I F Q R F T E G A Q D P S

1201 CTCGTCTAGTTGGTACAACCTGCATGAAGACTGGGCAGTCCGACTTTCC
L L D V M N V Y K Q G T L S F P

1301 CACAGCTGGCGGTCTAGCTACAGCTGCAAGACGTGGGTCCGGCTCTCG
T D V A L D I D V N Q V W A S L

1401 CGTGGAACAGGCCCACGTGCGCGGACATGTAGTCCGACTTCCGCGGGCG
R V K D P H L A S Y M L S F A G

1501 TATCAGGTGCGTCGCCAGCTACATCGGCAACTACATCAGCAGCTCGCA
Y D V C R D I Y G N I Y D D L T

1601 ACCGGCCACCGGTCGTCAGGGAACCTACCACCTCGCCAGAAGGACCTAC
Q G T A L L G K I T S R D E Q I

1701 CGACCCGAAGCCCGCGCGGGAGCCCAGCCAGTATCGCTATCTCCTGGT
P Q A E P A G E P R D Y R Y L V

1801 GAGCCGCAACTCGTCTAAGAGGTACGGGACTGGGTGGAACGCCGACAT
E A N L L N E M G Q G L K R S Y

S/D

1901 TAGCGACAGGAGACTAAGACAGACCACTCGGACGACAAGCTAGTCGGC

2001 GGAACCGCCGGCCCCGCCCCGCTGGACTCGCGTCACCGTCCGAACCACC
A K A A P A P S R L A T A P K T

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FIG. 3 (4/9)

GCTTCTAGGTAAAGAGGTACGCGTCCTTCAAGAACTGGAACAGGCCCCAGCAG
P F I W K E M R L F N K V K D P D D

GAACTCGCTGTCCTTCCAGGTCAAGTCGTCGAGTGGCCCCCTACAACGGGTAC
K L S L F T W N L L E G P I N G M

TCCAACAGGCCCCAGAACTTCAGCTGGCCGCACTGGTACCAGTGCTCCAACG
L N D P T K F D V P T V M T V L N

GGACTGTCTATGGCTCGTCTAACTGGAACAGCTGCGGCACCCGAAGCGCGTC
G Q C I G L L N V K D V G H A E R L

CTCGCGCGCCAGCGGGACTAGGCCTATCGGGGTCAGCTAGTCCATCTGCAGG
L A R D A Q D P Y G W D I L Y V D

AGCCAGTACATCCCGGCCAACTGGTCCCGCATCTGCTAGTCCTCGCCGTGCC
D T M Y P R N V L A Y V I L L P V

CGAACAGGAGCGACATCTGCCGGACGAACCCCGACTGCGGCCACAGCTACCG
L K D E S Y V A Q K P S V G T D I A

CAGCTCGTAGAGGACCCACTCAAACAGGCCCGCCTACAGCTACCCCTTGAC
D L M E Q T L K D P R I D I P F M

ORF3

GAACTGCGAGTTAGCCGGCCTAACCAGAACCACCACGCGGCCGCACGGCCTC
* D A P N T K T T R R R A P

GGCCGAGGAGTGGCCGGTCCCGACGCTGCGCTGCAAGGTCTAGCGCGTCCGA
A P E E G A L A A V R R E L D R L S

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FIG. 3 (6/9)

TCCCACGCGTCGAGCGACGCCCAGCCGTACAAGTTACGCGGGTCCAAGAGC
L T P L E S R T P M N L A G L N E

CGGCCGGCACCTACGCCCCGATAAGGCCACACCGCTGGAGCAGCCGTATCC
L R G H I R A Y E P T A V E D A Y

CTACCAAAACGGCGACAGCTACTGTGGGACCTACATCCTCGGGTACACGAG
I T K G S D I V G Q I Y S G M H E

CGCCGGACTATGAGGTACCGCGACGCGTAGTCGAGGACTATGACCGAGACG
A A Q Y E M A S R M L E Q Y Q S Q

ACAGGAACTTGACGAGCACCCCGTACAACGCGTCGTACAGCGGGCCGTAGC
S D K F Q E H P M N R L M D G P M

CTTGCTGTAGTCCGCGACGTACCACAACGGCGGCAGCAGCTCGCTCGGCGA
F S M L R Q M T N G G D D L S G S

AACGGCCGCAACGGGAGCAGCCGCGCAAACCGCTTCCACAGCCGCTCTGCC
N G A N G E D A R K A F T D A L R

CTAGGCCCCGCGCCCGCGGTACCGAAAGGTGGTGACTAGCTACCGGCGGA
L D P A A P A M A K W W Q D I A A

CGCCGAGTACAGCCAGGTCAACAGGGTCATGAACGCAACGTCAAGCTCGGT
R S M D T W N D W Y K R Q L E L W

← mRNA
GCGCACCACAACCCGCTTGCCATAGTACACCTAAACACGTGACGTTGTTTC

"-35" Ketothiolase

"-10" ORF2 "-35"

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FIG. 3 (7/9)

"-10"

3101 TACGCATCTTAGGTGTCCGGCGCTGGGGTATAGCCCCTGCGAGCAGGTA
V D A G R S A I G T F G G S L S
3201 GCAGCTGCGGCCGGCGTCACGGTAGCCTTGGAAGCCGCCGTCAGACAGC
R T G L A P E Q I D E V I L G Q V
3301 GCATGGCCTGAGCGCGGCCTTGTCTAGCTGCTCCACTAAGAGCCGGTCC
G L P H S V P A M T I N K V C G
3401 GCCCCGATGGCGTAAGCCACGGCCGGTACTGGTAGTTGTTCCAGACGCC
A D I V I A G G Q E S M S Q S S
3501 ACGGCTGTAGCAGTAGCGGCCGCCAGTCCTCTCGTACTCGGTCAGGAGC
K D T M I V D G L W D A F N N Y H
3601 TTCCTGTGGTACTAGCAGCTGCCGGAGACCCTACGGAAGTTGTTGATAG
Q Q D A F A A A S Q Q K T E A A
3701 TCGTCGTCCTGCGGAAGCGGCGGCGGAGCGTCGTCTTCTGGCTCCGGCG
R K G D P K V F D A D E F P R H
3801 CGCGTTCCCGCTAGGCTTCCACAACTACGGCTGCTCAAGGGCGCAGTG
G S V T A G N A S G I N D G A A M
3901 CCGTCGCAGTGCCGCCCATTTGCGGAGGCCGTAGTTGCTGCCCCGCCGGT
A R L V A F A S A G V D P A I M
4001 ACCGCGCAGACCACCGGAAGCGGTGCGGCCACAGCTAGGCCGCTAGTA

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FIG. 3 (8/9)

S/D Ketothiolase
M S E N I V I
AAGGTTAGTCTTAGGTCTCCTGTAGGTGCGGTACTCGCTCTTGTAGCAGTA
S L S A T E I G T A V L K G L L A
AGTGAGAGCCGGTGGCTCTAGCCGTGGCGGCACGAGTTCCCCGACGACCGC
L T A G V G Q N P A R Q T T L H A
ACGACTGGCGGCCCGCACCCGGTCTTGGGGCGGGCAGTCTGGTGCGACGTGC
S G L K A V H L A M Q A I A C G D
GTCGCCAGACTTCCGCCACGTAGACCGCTACGTCCGGTAGCGGACGCCCT
H V L P R S R D G Q R M G D W S M
GTGCAGGACGGCGCAAGCGCGCTGCCAGTCGCGTACCCGCTGACCAGCTAC
M G T T A E N I A Q K Y G F T R E
TGTACCCGTGGTGGCGGCTCTTGTAGCGGGTCTTCATGCCGAAATGCGCGC
Q K A G R F Q D E I I P I E I P Q
CGTCTTCCGACCGGCGAAGGTCCTGCTCTAGTAAGGCTAGCTCTAAGGCGT
G T T A E S L G K L R P A F S K D
CCGTGGTGCCGGCTCTCAGACCCGTTTCGACGCAGGCCGGAAGAGCTTCCTG
V V V M K E S K A K E L G L K P M
ACCAGCACCCTACTTCCTCAGGTTCCGGTTCCTTGACCCAGACTTCGGCT
G T G P I P A S T K C L E K A G W
CCCCTGCCCCGGGCTAGGGCCGCAGCTGGTTACGGACCTCTTCCGGCCGAC

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FIG. 3 (9/9)

T P A D L D L I E A N E A F A A
4101 CTGGGGCCGCCTAGACCTAGACTAGCTCCGGTTGCTTCGGAAGCGGCGC

V N G G A I A I G H P I G A S G A
4201 CAGTTGCCGCCGCGGTAGCGGTAGCCAGTAGGCTAGCCGCGGAGGCCAC

G L A T L C I G G G Q G V A L A
4301 TCCCAGACCGCTGCGACACGTAGCCGCCGCCGGTCCCGCACCGCGACCG

4401 TCGGAGGACTTAGCGAGGTCCGTGACTTGCGGGACGGCTAGGGCCTAGC

*Sma*I
4501 GGGCCC

FIG. 3

Q A M S V N Q D M G W D L S K V N
GTCCGGTACAGCCAGTTGGTCCTGTACCCGACCCTAGACAGGTTCCAGTTG

R V L V T L L Y E M Q K R D A K K
GCGCGCACGAGCACTGGGACGAGATACTCTACGTCTTCGCGCTGCGGTTCT

V E R M *
CCAGCTCGCCTACACTCGGCAGCAGGCGGCCAGACTTAGCGGCCGCCTGGC

CACCCCGCAAACGCGCGAACCCCATCTGAACGGCTTGCTGGTCGGCTTGGC

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FIG. 4 (1/3)

PHB polymerase	173	E	S	G	G	E	S	L	R	A	G	V	R	N	M	M	E	D	L	T	R	-	-	G	K	I	S	Q	T	D	E	S	A	F	E	V	-		
ORF 3	1	M	F	P	I	D	I	R	P	D	K	L	T	Q	E	M	L	D	Y	S	R	-	-	-	K	L	G	Q	G	M	E	N	L	L	N	A	E		
PHA polymerase	1 149	E	T	G	G	K	S	L	L	D	G	L	S	N	L	A	K	D	L	V	N	N	G	G	M	P	S	Q	V	N	M	D	A	F	E	V	-		
PHA polymerase	2 149	N	S	G	G	Q	S	L	V	F	G	V	A	H	L	L	D	D	L	R	H	N	D	G	L	P	P	Q	V	D	E	R	A	F	E	V	-		
PHB polymerase	206	G	R	N	V	A	V	T	E	G	A	V	V	F	E	N	E	Y	F	Q	L	Q	R	Y	Y	K	P	L	T	D	-	-	K	V	H	A	R	P	
ORF 3	34	A	I	D	T	G	V	S	P	K	Q	A	V	V	S	E	D	K	V	V	H	L	I	Q	Y	D	R	P	E	G	A	P	E	A	Q	P	P		
PHA polymerase	1 184	G	K	N	L	G	T	S	E	G	A	V	V	X	R	N	D	V	L	E	L	I	Q	Y	K	P	I	T	E	-	-	Q	V	H	A	R	P		
PHA polymerase	2 184	G	G	N	L	A	A	T	A	G	A	V	V	F	R	N	E	L	L	E	L	I	Q	Y	K	R	M	S	E	-	-	K	Q	H	A	R	P		
PHB polymerase	240	L	L	M	V	P	P	C	I	N	N	K	F	I	L	T	D	I	Q	P	E	S	S	L	V	R	H	V	V	E	Q	T	S	H	T	V	F	L	V
ORF 3	70	L	L	I	V	Y	A	L	V	I	N	K	F	I	L	T	D	I	Q	P	E	S	S	L	V	R	H	V	V	E	Q	T	S	H	T	V	F	L	V
PHA polymerase	1 218	L	L	I	V	Y	A	L	V	I	N	K	F	I	L	T	D	I	Q	P	E	S	S	L	V	R	H	V	V	E	Q	T	S	H	T	V	F	L	V
PHA polymerase	2 218	L	L	I	V	Y	A	L	V	I	N	K	F	I	L	T	D	I	Q	P	E	S	S	L	V	R	H	V	V	E	Q	T	S	H	T	V	F	L	V
PHB polymerase	276	S	W	R	N	P	D	A	S	M	A	G	S	T	W	D	D	Y	I	E	H	A	A	I	R	A	I	E	V	A	R	D	I	S	G	Q	V	D	D
ORF 3	106	D	W	G	Y	P	D	Q	A	D	R	A	L	T	L	D	D	Y	I	N	G	Y	I	D	R	C	V	E	L	L	R	E	A	H	T	G	G	V	D
PHA polymerase	1 254	S	W	R	N	P	T	K	A	Q	R	E	W	G	L	S	T	Y	I	D	-	A	L	K	E	A	V	D	A	V	L	A	I	S	G	G	V	D	
PHA polymerase	2 254	S	W	R	N	P	D	P	R	H	R	E	W	G	L	S	S	Y	V	Q	-	A	L	E	E	A	L	N	A	C	R	S	I	S	G	G	V	D	

FIG. 4(2/3)

PHB polymerase 312 K I N V L G F C V G G T I V S T A L A V L A A R G G E - H P A A S V T L L L
 ORF3 142 K V N I L G I C Q Q G G A F - - S L M Y S A L H P D - K V R N L V T L M V
 PHA polymerase 1288 D L N M L G A C C S G G I T C T A L V G H Y A A L A L G P D - N K V N A L T L L
 PHA polymerase 2288 D P N L M G A C C S G G L T M A A L Q G H L Q A K H Q L R R V R S A T Y L

PHB polymerase 347 T L L D F - A D T G I L D V F V D E G H V Q Q L R E A T L G G A G A P -
 ORF3 174 T P - V D F K T P D N L L S A W V Q N V D L A V D L M G N I P G E -
 PHA polymerase 1323 V S V L D T - T M D N Q V A L F V D E Q T L E A - - - A K R R S Y Q
 PHA polymerase 2324 V S L L D S - K F E S P A S L F A D E Q T I E A - - - A K R R S Y Q

PHB polymerase 382 C A T L R G L E L A N T F S F L R P N D L V W N Y V V D N Y L K G N T P
 ORF3 208 - L L N W T F L S L K P F S L T G Q K Y V N M V D L L L E D D P D K V K N F
 PHA polymerase 1353 A G V L E G S E M A K V F A W M R P N D L I W N Y W V N N Y L L G N E P
 PHA polymerase 2354 R G V L D G A E V A R I E E A W M R P N D L I W N Y W V N N Y L L G K T P

PHB polymerase 418 V P F D L L F W N G D A T N L P G P W Y C W Y L R H T Y L Q N N E L K V P
 ORF3 243 L R M E K - - W I F D S P D Q A G E T F R Q F I K D E Y Y Q N N G F - L N
 PHA polymerase 1389 P V F D I L F W N N D T T R L P A A F H G - D L L I E M F K S N P L T R P
 PHA polymerase 2390 P A F D I L Y W N N A D S T T R L P A A L H G - D L L D E F F K L N P L T H P

FIG. 4 (3/3)

PHB polymerase 454 G K L T V C G V P V D L A S I D V P T Y I Y G S R E D H I U P W T A A Y
 ORF3 276 C G V V L G C G Q E V D L K D I T C P V L N I F A L Q D H L V P P D A S R
 PHA polymerase 1 424 D A L E V C G T P I D L K Q K V K C D I Y S L A G T N D H I T P P W Q S C Y
 PHA polymerase 2 425 A G L E V C G T P I D L Q K V E L D S F T V A G S N D H I T P P W D A V

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PHB polymerase 490 A S T A L L L A N - - - K T R F V L G A S G - H I A G V I N P P A K N K
 ORF3 312 A L K G L T S S P D Y T E L A F F P G G H I G I Y V S K A Q K E V T P A
 PHA polymerase 1 460 R S A H L F G G - - - K I E F V L S N S G - H I Q S I L N P P G N P A
 PHA polymerase 2 461 R S A L L L G G - - - D R R F V L A N S G - H I Q S I L N P P G N P K

PHB polymertase 521 R S H W T N D A
 ORF3 348 I G K M L N E R
 PHA polymerase 1 491 A R F M T G A D
 PHA polymerase 1 492 A Y Y L A N P K

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FIG.5 (1/20)

EcoRI

1 GCTTAAGTAGTCCCGGTGATAGAGGTTGACGGCTCAAGCCTGAACTTGAA
101 CCGCGAAGTGTGCCCTGCCGACTAGGACTGAGGTAGGCTAGGCGTAGAGCG
201 TCACGAGGTAAGTACGACCTCTTAGGTGTGCGCCCCCGCGCTGGCTCGG
301 GGCAGGCCCTAAGCGCTACTGCAGTCTCGCCCCCGCAAGAGCGGTATAAGCGT
401 GGGTGGGGCTACACAGCCCCACAGCGCGGGGACAGCCCGACCGCGCGGA
501 ACGCCGCTCTGTGCAGGGGGACCGAGACCCCTTGCTCGTTCCTCCT
601 ACGGCTGCGGCTACAAGAAGCTCGTCGAGTAAGCCCCAGTCAACCCTAGCCGA
701 AATTGCACTTCCGGTCGAGGTTACAGGCGAGCTACTCGCTGCTCGGGCCAC
801 GCGGCCTTAACGAGGCCCGCGGCCCTTCGCAATAGCGAGCAACTCGGTGA
★ R E N L W

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FIG. 5 (2/20)

GCTAAAGGTCCTGGGACAGTGTAATTTGGGCTATCAGACGAGTACTCATG
CGGCCTACAGCTAAACAACCTAGCTTCTCTGACTCGGCCGGACCTAAGGAC
AAACGCTGACCGCGCGGCGAAGGACTCAGATGACTGGAAACGGCCGACGC
AGGGCGGTCAGTCAGTCGTAGGTTCCAACTACCAACCGGCATTGTGCGC
CCACCTTGTGACCCCGGTGCGTAGGCGTACGGCCGCCACGTTACAGGCCGG
AAGCCTGCGCGGAAGCACGTCCTAGCTTCTCCAGAAAGGCCCACTCCGTC
CCGCTGTAAAGATGTAGGTCCTAACTTAGTTCGGACCATATCCCCCGGG

TCAGGGCTCGCAGTAGCACCTACGGCAAGCTGCCGACTGCTGTCGCTCG

ATGGCTACCGCGCGCACTGAAGAAAGACGGGAAACGGGACTGTATCTA
K G I A P T V E K Q A K G S V Y I

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FIG. 5 (3/20)

901	CGGTACACTGGGGCCCTTCGGTCGAGCCACATCAGGCCGACG G H I G G P F A L E T Y D P S
1001	TCGGCTTCTACAACCTCGTGGCCCGTCCACTACAGGAAGTCCAGGTG L A F I N L V P C T I D K L D V
1101	TCAGGAAC TACTTGACTGCCTTCCAGAGCGCGGACTAGGCCGAC F D K I F Q R F T E G A Q D P S
1201	CTCGTCTAGTTGGTACAAC TGCATGAAGACTGGGAGTCCGACTTTC L L D V M N V Y K Q G T L S F
1301	CACAGCTGGGGTCTAGCTACAGCTGCAAGACGTGGTCCGGCTCTC T D V A L D I D V N Q V W A S L
1401	CGTGGAACAGGCCACGTCGGCGACATGTAGTCCGACTTCCGGCGG R V K D P H L A S Y M L S F A G
1501	TATCAGGTGCGTCGCCAGCTACATCGGCAACTACATCAGCAGCTCGC Y D V C R D I Y G N I Y D D L

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FIG. 5 (4/20)

ACCAGTCAGGGAACTCGGGGATCTCCGTAGGCCGCCGTGTCCTCCTAGGACC
S T L G K L A R S A D P P V L H D Q

AAGGACAGGGGTCGTGGTGGGGGTAACCTCCTTCGGCAACAAGACCATCT
E Q G G L V V G G N L F G N N Q Y

AGCTTCTAGGTAAGAGGTACGGTCCTTCAAGAACTGGAACAGGCCCAGCAG
P F I W K E M R L F N K V K D P D D

CGAACTCGCTGTCCCTTCAGGTCAAGTCGTGCGAGTGGCCCCCTACAACGGGTAC
P K L S L F T W N L L E G P I N G M

GTCCAACAGGGCCCCAGAACTTCAGCTGGCCGCACTGGTACCAGTGCTCCAACG
L N D P T K F D V P T V M T V L N

GGGACTGTCTATGGCTCGTCTAACTGGAACAGCTGGCGCACCCGAAGCGCGTC
G Q C I G L L N V K D V G H A E R L

ACTCGCGCGCCAGGGGACTAGGCCCTATCGGGGTCAGCTAGTCCATCTGCAGG
T L A R D A Q D P Y G W D I L Y V D

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FIG. 5 (5/20)

1601	ACCGCCACCGGTCGTCAGGGAACCTACCACCTCGCCAGAGGACCTA Q G T A L L G K I T S R D E Q I
1701	CGACCCGAAGCCCGCGGGAGCCAGCAGTATCGCTATCTCCTGG P Q A E P A G E P R D Y R Y L V
1801	GAGCCGCAACTCGTCTAAGAGGTACGGGACTGGGTGGAACGCCGACA E A N L L N E M G Q G L K R S
1901	TAGCGACAGGAGACTAAGACAGACCACCTCGGACGACAAGCTAGTCGG S/D
2001	GGAACCGCGCGCCCGCGCTGGACTCGCGTCACCGTCCGGAACCAC A K A A P A P S R L A T A P K T
2101	CACTGCGTCCCGGAACAAGAGCGCTGCCAGAGGACCTCGGCCAGGA H R L A K N E R R T E Q L R D
2201	AGCTGGTCCTAGCTGTACGCCGAAGAACTCGCGGTAGACTCGCAACTG D V L I S M R K K L A M Q A N V

FIG. 5^(6/20)

GTCTGGCCGGCACCTACGCCCGCATAGGCCACACCGCTGGAGCAGCCGTATCC
L R G H I R A Y E P T A V E D A Y

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FIG. 5 (7/20)

2301 GCGGAAGCGTCGTCGCGCTGGGTCAACAGCATCTCGCGCGCGGCTC
A G E C C A V W N D Y L A R A S

2401 CTGGCTGAAGTACGGCTCGAACCACATCTGCAACCACATAAGGACCT
V S K M G L K T Y V N T Y E Q

2501 AGGAGCGCCACATCGGCTCTGGCCCGGCTCTCCCGAGCTAGTTC
E E R T Y G L G P A S L A R D L

2601 CGCTGTAGCTCCTGTAGTACGGACGGTCAACAGCTCGCCGTCGAGG
P S M S S M M R Q W N D L P L E

2701 CTTGCGAAGACGTACAGAAGCTCGCAGAACCAGGTCTCGAGGTTCC
F R K Q M D E L T K T W L E L

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FIG.5 (8/20)

AGCTACCAAAACGGCGACAGCTACTGTGGGACCTACATCCTCGGTACACGAG
D I T K G S D I V G Q I Y S G M H E

CGGCCGGACTATGAGGTACCGGACGCGTAGTCGAGGACTATGACCGAGACG
L A A Q Y E M A S R M L E Q Y Q S Q

TGACAGGAACTTGACGAGCACCCCGTACAACGCGTCGTACAGCGGCGCGTAGC
S D K F Q E H P M N R L M D G P M

GTCTTGCTGTAGTCCGCGACGTACCACAACGGCGGCGACGAGCTCGCTCGGCGA
W F S M L R Q M T N G G D D L S G S

GCAACGGCCGCAACGGGAGCAGCGCGCAACCGCTTCCACAGCCGCTCTGCC
G N G A N G E D A R K A F T D A L R

FIG. 5_(9/20)

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2801 TTCTTCAAGAACGGACGAGGTAGTAAAGGTACTTGCTCGCACTGT
F F N K G Q E M M K E M F S R S

2901 GGTCCGCACACCGGCTTGACCTCCGGACAGGTCTGGTACCGAA
E W P T T A S S S A S D L G M A K

3001 ATCGTCATTGAAGAAATTAGTGTGCTCATGGACGTACCCTCCTCGTG
TAGCAGTAACCTCTTTAATCACAAACGAGTACCTGCATGGAGGAGCACC
D D N F F N T N S M S/D
ORF2

3101
ATGCGTAGAATCCACAGGCCGCCGACCCCATATCGGGACGCTCGTCCAT
TACGCTTCTAGGTGTCCGGCGCTGGGTATAGCCCCCTGCGAGCAGGTA

3201 V D A G R S A I G T F G G S L S
CGTCGACGGCGCGCAGTGCCATCGGAACCTTCGGCGGCAGTCTGTGCG

3301 R T G L A P E Q I D E V I L G Q V
CGTACCGGACTCGCGCGGAACAGATCGACGAGGTGATTCTCGGCCAGG

4401 G L P H S V P A M T I N K V C G
CGGGGCTACCGCATTCGGTGCCGGCCATGACCATCAACAAGGTCTGCGG

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FIG. 5^(10/20)

CTAGGCCCGCCGCGGTACCGAAGGTGGTACTAGTACCGCGGA 5'
 L D P A A P A M A K W W Q D I A A

CGCCGAGTACAGCCAGGTCAACAGGGTCATGAACGCAACGTCAAGCTCGGT 5'
 R S M D T W N D W Y K R Q L E L W

CGCGTGGTGTGGCGAAGGTATCATGTGGATTGTGCACTGCAACAAG 3'
 GCGCACCAACAACCGCTTGCCATAGTACACCTAAACACGTGACGTTGTTTC 5'
 "10" "35"
 mRNA

phbA →
 S/D M S E N I V I
 TTCCAATCAGAAATCCAGAGGACATCCACGCCCATGAGCGAGAACATCGTCAT 3'
 AAGGTTAGTCTTAGGTCTCCTGTAGTGCGGTACTCGCTCTTGTAGCAGTA 5'

S L S A T E I G T A V L K G L L A
 TCACTCTCGGCCACCGAGATCGGCACCGCGGTGCTCAAGGGGTGCTGGCG 3'

L T A G V G Q N P A R Q T T L H A
 TGCTGACCGCGCGGTGGGCCAGAACCCCGCGTCAGACCAACGCTGCACG 3'

S G L K A V H L A M Q A I A C G D
 CAGCGGTCTGAAGCGGTGCATCTGGCGATGCAGGCCCATCGCCTGCGGGGA

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FIG. 5 (11/20)

3501 A D I V I A G G Q E S M S Q S S H
TGCCGACATCGTCATCGCCGGCGGTCAGGAGAGCATGAGCCAGTCCTCGC

3601 K D T M I V D G L W D A F N N Y H
AAGGACACCATGATCGTCGACGGCCTCTGGGATGCCCTTCAACAATATCA

3701 Q Q D A F A A S Q Q K T E A A
AGCAGCAGGACGCCTTCGCCGCCCTCGCAGCAGAAGACCGAGGCCCGC

3801 R K G D P K V F D A D E F P R H G
GCGCAAGGGCGATCCGAAGGTGTTGATGCCGACGAGTTCCTCCGCGTCACG

3901 G S V T A G N A S G I N D G A A M
GGCAGCGTCACGGCGGTAACGCCCTCCGGCATCAACGACGGGGGGCCAT

4001 A R L V A F A S A G V D P A I M
TGGCGCGTCTGGTGGCCTTCGCCAGCGCGGTGTCGATCCGGCGATCATG

4101 T P A D L D L I E A N E A F A A Q
GACCCCGGGGATCTGGATCTGATCGAGGCCAACGAAGCCTTCGCCCGGC

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FIG. 5 (12/20)

V L P R S R D G Q R M G D W S M
ACGTCCTGCCGCGTTCCGCGACGGTCAGCGCATGGCGACTGGTCGATG

M G T T A E N I A Q K Y G F T R E
CATGGCACCAACCGCGAGAACATCGCCAGAACTACGGCTTACGGCGG

Q K A G R F Q D E I I P I E I P Q
CAGAAGGCTGGCGCTTCCAGGACGAGATCATTCGGATCGAGATTCCGCA

T T A E S L G K L R P A F S K D
GCACCAGGCCGAGAGTCTGGGCAAGCTGGTGCCGGCCTTCTCGAAGGAC

V V V M K E S K A K E L G L K P M
GGTCGTGGTGATGAAGGAGTCCCAAGGCCAAGGAAGTGGGTCTGAAGCCGA

G T G P I P A S T K C L E K A G W
GGGACGGGCCGATCCCGCGTCCGACCAAGTGCCTGGAGAAGGCCGGCTG

A M S V N Q D M G W D L S K V N
AGGCCATGTCGGTCAACCAGGACATGGGCTGGGATCTGTCCAAAGGTCAAC

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FIG. 5 (13/20)

4201 V N G G A I A I G H P I G A S G
GTCAACGGCGCGCCATCGCCATCGGTGCATCCGATCGGCGCCTCCGG

4301 G L A T L C I G G Q G V A L
AGGTCTGGGACGCTGTGCATCGGCGCGGCCAGGGCGTGGCGCTG

4401 AGCCTCCTGAATCGCTCCAGGCACTGAACGCCCTGCCGATCCCCGAT

ORF4 →

4501 SmaI M N S E R I I K K Y P N
CCGGGTGCCCATGAACAGCGAGCGCATCATCAAGAAGTATCCGAAC

4601 D L V M S G Q P F R V L D S A N
CGATCTGGTGATGAGCGGACAGCCCTTCGGCTCCTCGACAGCGCCA

4701 E T G G Q P L F S A N M L A Q I
ACGGCGGTACGCCGCTGTTTCAGCGCCCAACATGCTGGCCCATCAT

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FIG. 5 (14/20)

A R V L V T L L Y E M Q K R D A K K
 TGCGCGGTGCTCGTGACCCCTGCTCTATGAGATGCAGAGCGGACGCCAAGA

A V E R M *
 GCGGTCGAGCGGATGTGAGCCGTCGTCGCCGGTCTGAATCGCGGGACCG

CGGTGGGGCGTTTGCGCGCTTGGGGTAGACTTGCCGAACGACCGCAACCG

R R L Y D T E V S R Y I T L A D V R
 CGCCGCTCTACGACACCGAGGTCAGCCGCTATATCACCCCTGCCGATGTGG

D S D I T R S I L L Q I M L E E E
 ATGACAGGATATCACCCGTTCCATCCTGCTCCAGATCATGCTGGAGGAGAG

I R F Y G G T L Q G T F A R Y L E S
 CCGCTTCTACGGCGGACCCCTTCAGGGCACCTTCGCCCGCTATCTGGAATCTT

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FIG. 5
(15/20)

4801	S L D L F A K Q Q Q E V T K A CACTCGACCTGTTCGCCAAGCAGCAACAGGAAGTGACCAAGGCACTC
4901	I W A D L Q D E L M R A A G F P CTGGGCTGATCTCCAGGACGAACTCATGCGCGGCTGGCTTCCGG
5001	GCCTCGGTCACAGCTTATTGTGCAATGCAACATTGCTGCACTGCA "-10" (?)
5101	E W T N K S V E R M T S F G E ACGAGTGGACCAACAAGAGCGTCGAGCGCATGACCACTTCGGTGAG
5201	L Y M D H S M R L M K L A T E S CCTGTACATGGATCACAGCATGCGCCTGATGAAGCTGGCCACCGAGT
5301	S E R V M A E S K A T M Q F F G AGGAGCGCGTCATGGCCGAGAGCAAGGCCACCATGCAGTTCTTCGG
5401	E D L R K S V A V * GCGAAGATCTGCGCAAGAGCGTCGCCGTCTAAAGACGCCGACCTCTG

L T D N P F G T V T R L T Q K N V E **FIG. 5** (16/20)
 ACCGACAATCCCTTCGGACGGTGACACGCCCTGACTCAGAAGAACGTCGAGAT

V A P R K K E *
 TCGCGCCGCGCAAGAAAAGAAATAATGAGGATTGCGAAATTGCGCTTGACG
 "-35" (?)

ORF5 →
 S/D
 M N T T D S L K T V N
 CAAACCTTACGGAGATGATCATGAACACCACCGACAGCCTCAAGACCGTCA

L N V R L F E K L A A R Q M D A V N
 CTGAACGTGCGTCTGTTCGAGAAGCTGGCCGCCGTCAGATGGACGCCGTGAA

K G Y N D L F K G Q V D A T K E L
 CCAAGGGTTACAATGACCTCTTCAAGGGTCAGGTCGACGCCACCAAGGAAC TG

D A R D E Y R V W F E K S L N D V S
 CGATGCCCCGCGACGAATACCGCGTGTGTTCCGAGAAGAGCCTGAACGACGTC A

GGCCATCGCGATCCAGGGATGGATCGCCATTGGTCATGCTTCGGATCGGCCG

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FIG. 5 (17/20)

5501 GGAGCAGCGCCCAATGGAACCAACGCTTACCTTGCCCTGCCCGCTTGGA

5601 AAGATTCCTG GAGGA S/D M A R I A L V T G G
 phbB →

phbg —————→

S/D

M A R I A L V T G C

5701 T V V A N C H P S E A A A E E
CACCGTCGTGGCGAACTGCCATCCGTCCGAGCGCGCCGCCGAAGA

[illegible]

5801 D V S S F D D S A R M V R E I T
GACGTGTCCTCGTTCGACGACAGCGCGCATGTTCCGCGATCACA

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100. 101. 102. 103. 104. 105. 106. 107. 108. 109. 110. 111. 112. 113. 114. 115. 116. 117. 118. 119. 120. 121. 122. 123. 124. 125. 126. 127. 128. 129. 130. 131. 132. 133. 134. 135. 136. 137. 138. 139. 140. 141. 142. 143. 144. 145. 146. 147. 148. 149. 150. 151. 152. 153. 154. 155. 156. 157. 158. 159. 160. 161. 162. 163. 164. 165. 166. 167. 168. 169. 170. 171. 172. 173. 174. 175. 176. 177. 178. 179. 180. 181. 182. 183. 184. 185. 186. 187. 188. 189. 190. 191. 192. 193. 194. 195. 196. 197. 198. 199. 200. 201. 202. 203. 204. 205. 206. 207. 208. 209. 210. 211. 212. 213. 214. 215. 216. 217. 218. 219. 220. 221. 222. 223. 224. 225. 226. 227. 228. 229. 230. 231. 232. 233. 234. 235. 236. 237. 238. 239. 240. 241. 242. 243. 244. 245. 246. 247. 248. 249. 250. 251. 252. 253. 254. 255. 256. 257. 258. 259. 260. 261. 262. 263. 264. 265. 266. 267. 268. 269. 270. 271. 272. 273. 274. 275. 276. 277. 278. 279. 280. 281. 282. 283. 284. 285. 286. 287. 288. 289. 290. 291. 292. 293. 294. 295. 296. 297. 298. 299. 300. 301. 302. 303. 304. 305. 306. 307. 308. 309. 310. 311. 312. 313. 314. 315. 316. 317. 318. 319. 320. 321. 322. 323. 324. 325. 326. 327. 328. 329. 330. 331. 332. 333. 334. 335. 336. 337. 338. 339. 340. 341. 342. 343. 344. 345. 346. 347. 348. 349. 350. 351. 352. 353. 354. 355. 356. 357. 358. 359. 360. 361. 362. 363. 364. 365. 366. 367. 368. 369. 370. 371. 372. 373. 374. 375. 376. 377. 378. 379. 380. 381. 382. 383. 384. 385. 386. 387. 388. 389. 390. 391. 392. 393. 394. 395. 396. 397. 398. 399. 400. 401. 402. 403. 404. 405. 406. 407. 408. 409. 410. 411. 412. 413. 414. 415. 416. 417. 418. 419. 420. 421. 422. 423. 424. 425. 426. 427. 428. 429. 430. 431. 432. 433. 434. 435. 436. 437. 438. 439. 440. 441. 442. 443. 444. 445. 446. 447. 448. 449. 450. 451. 452. 453. 454. 455. 456. 457. 458. 459. 460. 461. 462. 463. 464. 465. 466. 467. 468. 469. 470. 471. 472. 473. 474. 475. 476. 477. 478. 479. 480. 481. 482. 483. 484. 485. 486. 487. 488. 489. 490. 491. 492. 493. 494. 495. 496. 497. 498. 499. 500. 501. 502. 503. 504. 505. 506. 507. 508. 509. 510. 511. 512. 513. 514. 515. 516. 517. 518. 519. 520. 521. 522. 523. 524. 525. 526. 527. 528. 529. 530. 531. 532. 533. 534. 535. 536. 537. 538. 539. 540. 541. 542. 543. 544. 545. 546. 547. 548. 549. 550. 551. 552. 553. 554. 555. 556. 557. 558. 559. 560. 561. 562. 563. 564. 565. 566. 567. 568. 569. 570. 571. 572. 573. 574. 575. 576. 577. 578. 579. 580. 581. 582. 583. 584. 585. 586. 587. 588. 589. 590. 591. 592. 593. 594. 595. 596. 597. 598. 599. 600. 601. 602. 603. 604. 605. 606. 607. 608. 609. 610. 611. 612. 613. 614. 615. 616. 617. 618. 619. 620. 621. 622. 623. 624. 625. 626. 627. 628. 629. 630. 631. 632. 633. 634. 635. 636. 637. 638. 639. 640. 641. 642. 643. 644. 645. 646. 647. 648. 649. 650. 651. 652. 653. 654. 655. 656. 657. 658. 659. 660. 661. 662. 663. 664. 665. 666. 667. 668. 669. 670. 671. 672. 673. 674. 675. 676. 677. 678. 679. 680. 681. 682. 683. 684. 685. 686. 687. 688. 689. 690. 691. 692. 693. 694. 695. 696. 697. 698. 699. 700. 701. 702. 703. 704. 705. 706. 707. 708. 709. 710. 711. 712. 713. 714. 715. 716. 717. 718. 719. 720. 721. 722. 723. 724. 725. 726. 727. 728. 729. 730. 731. 732. 733. 734. 735. 736. 737. 738. 739. 740. 741. 742. 743. 744. 745. 746. 747. 748. 749. 750. 751. 752. 753. 754. 755. 756. 757. 758. 759. 760. 761. 762. 763. 764. 765. 766. 767. 768. 769. 770. 771. 772. 773. 774. 775. 776. 777. 778. 779. 780. 781. 782. 783. 784. 785. 786. 787. 788. 789. 790. 791. 792. 793. 794. 795. 796. 797. 798. 799. 800. 801. 802. 803. 804. 805. 806. 807. 808. 809. 810. 811. 812. 813. 814. 815. 816. 817. 818. 819. 820. 821. 822. 823. 824. 825. 826. 827. 828. 829. 830. 831. 832. 833. 834. 835. 836. 837. 838. 839. 840. 84

5901 K T F K K M E Q A H W E A V I N
ACAAGACCTTCAAGAAGATGGAGCAGGCGCACTGGGAGGCCGTGATCA

ACAGACCTTCAACAATCCACCGGCGGGCAGAAV IN

6001 L E R G F G R I I N I S S V N G
GCTGGAGCGCGGCTTCGGGGGTATCATCAACATCTCGTCGGTCAACGG

E K G F U R I N S V N G

6101 H G F T M A L A Q E G A S K G V
CACGGCTTCACCATGGCTCTGGCTCAGGAGGTGGTCCAAAGGGCGTG

U S F I M A L A Q E G A S K G V

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FIG. 5 (18/20)

GTAAAGTGGCCTTGAAGTTCGACGACACTGTTTCATCGTTCTCAATAGTTCCA

I G G I G T S I C T R L A K D G C
GCATCGGCGGCATCGGCACTTCGATCTGCACACGCCCTGGCAAAGGATGGCTG

W K Q A R A A E G F D I A V F T A
GTGGAAGCAGGCCCGTGCCGCCGAGGGTTTCGACATCGCCGTCTTCACCGCT

E Q V G P I D I L V N C A G I T R D
GAGCAGGTGCGTCCCATCGACATCCTGGTCAACTGTGCCGGCATCACCCGGC

V N L N S V F N V T R Q V W D G M
ACGTCAACCTCAACAGCGTCTTCAAGTCAACCCGTCAGGTGTGGGACGGGAT

Q R G Q F G Q A N Y S A A K A G M
TCAGCGGGCCAGTTCGGTCAAGCCCACTATTCCGCCGCCCAAGCCGGTATG

T V N T I S P G Y V E T A M T L A M
ACCGTCAACACCATCTCGCCCGGCTATGTCGAGACGGCCATGACCCCTGGCGA

FIG. 5^(19/20)

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6901 L A N G L L W A A I G S A H D A
CTGGCCAATGGTCTGCTGTGGCGCGGATTGGCTCAGCCCATGACG

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FIG.5
(20/20)

R M A Q P N E I A A A I A F L A G D
TCGCATGGCTCAGCCTAATGAGATCGCCGCCGCATCGCTTTCCTGGCCGGCGA

L F M H *

CTGTTTCATGCATGATTAGATCATACCGGGCCGAATACAAACACTGACAATG

ATGAGACGTTTACAGCCCGGCCAGCCGGGCTTTTGTGTAGAATCGAAT

R Y C D D V L D A A R F A D Y A P N
CGCTACTGCGATGACGTGCTCGACGGCGGCGCTTCGCCGACTATGCCCGGAAT

V T A S A A L I E A A I A E H A D A
GCGTGACGGCCAGCGCGGTGATCGAGGCCGCGATCGCGGAGCACGCCGACG

L I G I K G Q R A R T L L S A G V S
CCTGATCGGCATCAAGGGCAGCGCGCAAGGACATTGCTCAGCGGGGTGTGAG

N N A T L G R R L D F I D M E P T A
AACAATGCCACACTCGGTGCGCGCTCGATTTCATCGACATGGAACCGACCGCA

C V L H G A C L A
CCTGCGTCCCTTCAGGAGCATGTCTCGCATC

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FIG.6 (2/4)

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|------|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C.v. | 137 | - | D | W | K | D | T | M | I | V | D | G | L | W | D | A | F | N | N | Y | H | M | G | T | T | A | E | N | I | V | V | L | A | A | A | Q | K | E | Q | M | D | W | Y | N | I | | |
| A.e. | 136 | - | D | A | K | L | V | D | T | M | I | V | D | G | L | W | D | V | Y | N | Q | Y | H | M | G | T | T | A | E | N | I | V | V | L | A | A | A | Q | K | E | Q | M | D | W | Y | N | I |
| Z.r. | 136 | - | D | F | K | M | I | D | T | M | I | K | D | G | L | T | D | A | F | Y | G | Y | H | M | G | T | T | A | E | N | I | V | V | L | A | A | A | Q | K | E | Q | M | D | W | Y | N | I |
| E.c. | 135 | - | G | L | S | - | R | N | V | A | K | A | A | G | M | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |
| S.u. | 139 | - | Q | T | V | L | I | D | G | V | E | R | D | G | L | N | D | A | Y | D | G | L | A | M | G | V | H | A | E | N | I | V | V | L | A | A | A | Q | K | E | Q | M | D | W | Y | N | I |
| R.n. | 140 | L | D | L | K | L | E | D | T | L | W | - | A | G | L | D | T | Q | H | V | K | L | P | M | E | M | T | A | E | N | I | V | V | L | A | A | A | Q | K | E | Q | M | D | W | Y | N | I |

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|------|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C.v. | 172 | T | R | E | Q | Q | D | A | F | A | A | A | S | Q | Q | K | T | E | A | A | Q | K | A | G | R | F | Q | D | E | I | I | P | I | F | I | P | Q | |
| A.e. | 171 | T | R | E | A | Q | D | E | F | A | V | G | S | Q | N | K | A | E | A | A | Q | K | A | G | K | F | Q | D | E | I | I | P | I | F | I | P | Q | |
| Z.r. | 171 | S | R | D | E | Q | D | A | F | A | V | A | S | Q | N | K | A | E | A | A | Q | K | A | G | K | F | Q | D | E | I | I | P | I | F | I | P | Q | |
| E.c. | 161 | S | R | E | M | Q | D | A | F | A | A | R | S | H | A | R | A | W | A | A | T | Q | S | A | A | F | F | K | N | E | I | I | P | I | F | I | P | Q |
| S.u. | 174 | T | R | D | Q | Q | D | S | F | A | I | E | S | Y | Q | K | S | Q | Q | S | Q | K | E | G | K | F | Q | D | E | I | I | P | I | F | I | P | Q | |
| R.n. | 175 | S | R | E | D | C | D | R | Y | A | L | Q | S | Q | Q | R | W | K | A | A | N | E | A | G | Y | F | Q | D | E | I | I | P | I | F | I | P | Q | |

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|------|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C.v. | 208 | R | K | G | D | P | K | V | F | D | A | D | E | F | P | R | H | G | T | T | A | L | D | S | M | A | K | L | R | R | P | A | F | - | S | K | K | A | - | D | D | P | Q | R | E | - |
| A.e. | 207 | R | K | G | D | P | K | V | F | D | A | D | E | F | P | R | H | G | T | T | A | L | D | S | M | A | K | L | R | R | P | A | F | - | S | K | K | A | - | D | D | P | Q | R | E | - |
| Z.r. | 207 | R | K | G | D | P | K | V | F | D | A | D | E | F | P | R | H | G | T | T | A | L | D | S | M | A | K | L | R | R | P | A | F | - | S | K | K | A | - | D | D | P | Q | R | E | - |
| E.c. | 197 | A | D | G | V | L | K | Q | E | N | Y | D | E | F | P | R | H | G | T | T | A | L | D | S | M | A | K | L | R | R | P | A | F | - | S | K | K | A | - | D | D | P | Q | R | E | - |
| S.u. | 210 | F | R | G | K | P | D | T | Q | V | T | N | D | E | E | P | A | R | L | H | V | L | E | Q | L | A | K | L | R | R | P | A | F | - | S | K | K | A | - | D | D | P | Q | R | E | - |
| R.n. | 211 | K | K | C | K | Q | T | M | - | Q | V | D | E | H | A | R | P | Q | T | T | A | L | D | S | M | A | K | L | R | R | P | A | F | - | S | K | K | A | - | D | D | P | Q | R | E | - |

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FIG.6 (3/4)

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|------|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C.v. | 242 | V | T | A | G | N | A | S | G | I | N | D | G | A | A | M | V | V | M | K | E | S | K | A | K | E | L | G | L | K | P | M | A | R | L | |
| A.e. | 241 | T | V | T | A | G | N | A | S | G | L | N | D | G | A | A | A | V | V | M | S | A | A | K | A | K | E | L | G | L | T | P | L | A | T | I |
| Z.r. | 240 | T | V | T | A | G | N | A | S | G | L | N | D | G | A | A | A | V | V | M | S | A | A | K | A | K | E | L | G | L | T | P | L | A | T | I |
| E.c. | 232 | M | V | T | A | G | N | A | S | G | L | N | D | G | A | A | A | A | L | M | S | E | A | S | R | R | G | L | K | P | P | R | A | R | V | |
| S.u. | 245 | T | V | T | A | G | N | A | S | G | L | N | D | G | A | A | A | A | L | M | S | E | S | R | R | G | L | K | P | P | R | A | R | V | | |
| R.n. | 244 | T | V | T | A | G | N | A | S | G | L | N | D | G | A | A | A | A | L | M | S | E | S | R | R | G | L | K | P | P | R | A | R | V | | |

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|------|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C.v. | 278 | V | A | F | A | S | A | N | T | V | V | G | C | D | P | A | I | M | G | T | G | P | I | P | A | S | T | K | C | L | E | K | A | G | G | T | P |
| A.e. | 277 | K | S | Y | A | A | T | V | V | G | C | D | P | P | P | K | V | M | G | T | G | P | I | P | A | S | T | K | C | L | E | K | A | G | G | T | P |
| Z.r. | 276 | V | S | W | A | A | V | V | G | C | D | P | P | P | P | K | V | M | G | T | G | P | I | P | A | S | T | K | C | L | E | K | A | G | G | T | P |
| E.c. | 268 | R | S | M | A | A | V | V | G | C | D | P | P | P | P | S | I | M | G | T | G | P | I | P | A | S | T | K | C | L | E | K | A | G | G | T | P |
| S.u. | 281 | K | G | W | G | E | A | A | H | L | P | A | D | F | T | W | A | P | S | L | A | V | P | A | S | T | K | C | L | E | K | A | G | G | T | P | |
| R.n. | 280 | V | G | Y | F | V | S | G | C | D | P | A | I | M | G | T | G | P | I | P | A | S | T | K | C | L | E | K | A | G | G | T | P | A | Q | D | D |

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|------|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C.v. | 313 | D | L | I | E | A | N | E | A | F | A | A | Q | A | M | S | V | N | Q | D | M | G | W | D | L | - | - | - | - | - | - | - | - | - | - | - | - |
| A.e. | 312 | D | L | I | E | A | N | E | A | F | A | A | Q | A | M | S | V | N | Q | D | M | G | W | D | L | - | - | - | - | - | - | - | - | - | - | - | |
| Z.r. | 311 | D | L | I | E | A | N | E | A | F | A | A | Q | A | M | S | V | N | Q | D | M | G | W | D | L | - | - | - | - | - | - | - | - | - | - | - | |
| E.c. | 303 | G | V | F | E | M | N | E | A | F | A | A | Q | A | M | S | V | N | Q | D | M | G | W | D | L | - | - | - | - | - | - | - | - | - | - | - | |
| S.u. | 317 | D | Y | F | E | M | N | E | A | F | A | A | Q | A | M | S | V | N | Q | D | M | G | W | D | L | - | - | - | - | - | - | - | - | - | - | - | |
| R.n. | 315 | D | L | I | E | A | N | E | A | F | A | A | Q | A | M | S | V | N | Q | D | M | G | W | D | L | - | - | - | - | - | - | - | - | - | - | - | |

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FIG. 7 (1/2)

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|------|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C.v. | 1 | M | A | R | I | A | L | V | T | G | G | I | G | T | S | I | C | T | R | L | A | K | D | G | C | T | V | V | A | N | G | C | H | P | | |
| A.e. | 1 | M | T | Q | R | I | A | Y | V | T | G | G | M | G | I | G | T | A | I | C | Q | R | L | A | K | D | G | F | R | V | V | A | G | C | | |
| Z.r. | 1 | M | S | R | V | A | L | V | T | G | G | S | R | G | I | G | A | A | I | S | I | A | L | K | A | A | G | Y | K | V | A | R | S | Y | | |
| C.v. | 36 | S | E | A | A | A | E | E | W | K | Q | A | R | A | A | E | G | F | D | I | A | V | F | T | A | - | - | - | - | - | - | - | - | - | | |
| A.e. | 37 | - | N | S | P | R | R | E | K | W | L | E | Q | Q | K | A | L | G | F | D | F | I | A | S | E | G | - | - | - | - | - | - | - | - | | |
| Z.r. | 36 | - | N | D | - | - | - | - | - | - | - | D | A | A | K | P | - | - | - | - | F | K | A | E | T | G | I | A | V | Y | K | W | D | V | | |
| C.v. | 66 | D | S | A | R | M | V | R | E | I | T | E | Q | V | G | E | I | D | I | L | V | N | C | A | G | I | T | T | R | R | K | T | F | K | | |
| A.e. | 66 | D | S | T | K | T | A | F | D | K | V | K | S | E | V | G | E | V | D | V | L | I | N | N | A | G | I | T | T | R | D | V | V | F | K | |
| Z.r. | 60 | E | A | C | V | E | G | I | A | K | V | E | A | D | L | G | P | I | D | V | L | V | N | N | A | G | I | T | K | D | A | M | F | K | | |
| C.v. | 102 | Q | A | H | E | A | V | I | N | V | N | L | N | T | S | V | F | N | V | T | R | Q | Y | W | D | G | M | L | E | R | G | F | G | R | I | |
| A.e. | 102 | R | A | D | N | D | A | V | I | N | V | N | L | N | T | S | L | F | N | V | T | K | Q | V | I | D | G | M | A | D | R | G | W | G | R | I |
| Z.r. | 96 | P | D | Q | A | N | A | V | I | N | T | N | L | N | T | S | L | F | N | M | T | H | P | V | N | S | G | M | R | D | R | S | F | G | R | I |

FIG. 7 (2/2)

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| | | | | |
|------|-----|-------------------------------------|--|---------------------------------|
| C.v. | 138 | I S S V N G Q Q R | G Q F G Q A N Y S A A K A G | M H G F F T M A L A Q E E G A S |
| A.e. | 138 | I S S V N G Q Q K | G Q F G Q A N Y S A A K A G | L H G F F T M A L A Q E E V A T |
| Z.r. | 132 | I S S I N G Q Q K | G Q F G Q A N Y S A A K A G | D L G F F T K A L A Q E E G A A |
| C.v. | 174 | K G V T V N T I S P G G Y | V E T A M T L A M N D D | D V R - N S I I S G I P M |
| A.e. | 174 | K G V T V N T I S P G G Y | V E T A M T L A M N D D | D V R - N S I I S G I P M |
| Z.r. | 168 | K G I T V N A I C P G G Y | I G T E M V R A I P E K V | L N E R I T P Q I P V |
| C.v. | 209 | R M A Q P N E I A A A I A F L A G | D E S S G Y M T G A N L P V | N G G L F E |
| A.e. | 209 | R M A Q P N E I A A A I A F L A G | D E S S G Y M T G A N L P V | N G G L F E |
| Z.r. | 204 | G L R G E P D E I A R I V V F L A S | D E A G F I T G S T I S A | N G G L F E |
| C.v. | 245 | M H | Homology to reductase of <i>C. vinosum</i> : | |
| A.e. | 245 | M G | 56.4% | |
| Z.r. | 240 | F V | 48.3% | |

Comparison of amino acid sequences of reductases encoded by
C. vinosum (C.v.), *A. eutrophus* (A.e.) and *Z. ramifera* (Z.r.).

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FIG. 8 (1/2)

| Strain (plasmid) | relevant markers | Medium |
|---|--|------------|
| S17-1 (pHP1014) | none | LB-Tc-Glu |
| S17-1 (pHP1014::PP10) | <i>phbA</i> ⁺ , <i>phbB</i> ⁺ , <i>phbC</i> ⁺ , ORF2 ⁺ | LB-Tc-Glu |
| S17-1 (pHP1014::EP94) | <i>phbA</i> ⁺ , <i>phbB</i> ⁺ , <i>phbC</i> ⁺ , ORF2 ⁺ | LB-Tc-Glu |
| S17-1 (pSUP202) | none | LB-Tc-Glu |
| S17-1 (pSUP202::PP10) | <i>phbA</i> ⁺ , <i>phbB</i> ⁺ , <i>phbC</i> ⁺ , ORF2 ⁺ | LB-Tc-Glu |
| XL1-Blue (KS ⁻) | none | LB-Ap-Glu |
| XL1-Blue (KS ⁻) | none | LB-Ap-IPTG |
| XL1-Blue (KS ⁻ ::SE45 ⁺) | <i>phbA</i> ⁺ , <i>phbB</i> ⁻ , <i>phbC</i> ⁺ , ORF2 ⁺ | LB-Ap-Glu |
| XL1-Blue (KS ⁻ ::SE45 ⁺) | <i>phbA</i> ⁺ , <i>phbB</i> ⁻ , <i>phbC</i> ⁺ , ORF2 ⁺ | LB-Ap-IPTG |
| XL1-Blue (KS ⁻ ::SE45 ⁻) | <i>phbA</i> ⁺ , <i>phbB</i> ⁻ , <i>phbC</i> ⁺ , ORF2 ⁺ | LB-Ap-Glu |
| XL1-Blue (KS ⁻ ::SE45 ⁻) | <i>phbA</i> ⁺ , <i>phbB</i> ⁻ , <i>phbC</i> ⁺ , ORF2 ⁺ | LB-Ap-IPTG |
| XL1-Blue (KS ⁻ ::B55) | <i>phbA</i> ⁺ , <i>phbB</i> ⁺ , <i>phbC</i> ⁻ , ORF2 ⁺ | LB-Ap-Glu |
| XL1-Blue (KS ⁻ ::B55) | <i>phbA</i> ⁺ , <i>phbB</i> ⁺ , <i>phbC</i> ⁻ , ORF2 ⁺ | LB-Ap-IPTG |

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FIG. 8 (2/2)

| Specific activity (U/g of protein) | | | | Accumulation of |
|------------------------------------|-----------------------|---------------------------|-----------------|-----------------------------------|
| PHB synthase | β -Ketothiolase | Acetoacetyl-CoA reductase | | PHB
(% of cellular dry weight) |
| | | NADH-dependent | NADPH-dependent | |
| <0.1 | <20 | <20 | <20 | <0.1 |
| 6.1 | 1190 | 420 | 60 | 10.2 |
| 5.2 | 940 | 310 | 60 | 9.7 |
| <0.1 | <20 | <20 | <20 | <0.1 |
| 6.1 | 1320 | 320 | 60 | 12.1 |
| <0.1 | <20 | <20 | <20 | <0.1 |
| <0.1 | <20 | <20 | <20 | <0.1 |
| 6.0 | 1120 | <20 | <20 | <0.1 |
| 4.3 | 980 | <20 | <20 | <0.1 |
| 4.8 | 470 | <20 | <20 | <0.1 |
| 2.9 | 70 | <20 | <20 | <0.1 |
| <0.1 | 80 | 30 | <20 | <0.1 |
| <0.1 | 1870 | 610 | 40 | <0.1 |

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 92/01291

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/52; C12N15/53; C12N15/54; C12N1/21
 A01H5/00; C12P7/62

II. FIELDS SEARCHEDMinimum Documentation Searched⁷

Classification System

Classification Symbols

Int.Cl. 5

C12N ; C12P ; A01H

Documentation Searched other than Minimum Documentation
 to the Extent that such Documents are included in the Fields Searched⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

| Category ¹⁰ | Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ |
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| A | WO,A,9 100 917 (MIT)
24 January 1991
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| | ---
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¹⁰ Special categories of cited documents:¹⁰ "A" document defining the general state of the art which is not considered to be of particular relevance¹⁰ "E" earlier document but published on or after the international filing date¹⁰ "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)¹⁰ "O" document referring to an oral disclosure, use, exhibition or other means¹⁰ "P" document published prior to the international filing date but later than the priority date claimed¹⁰ "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention¹⁰ "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step¹⁰ "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.¹⁰ "A" document member of the same patent family**IV. CERTIFICATION**

Date of the Actual Completion of the International Search

09 OCTOBER 1992

Date of Mailing of this International Search Report

19. 10. 92

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

MADDOX A.D.

III. DOCUMENTS CONSIDERED TO BE RELEVANT

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ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. GB 9201291
SA 62148

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 09/10/92

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date | Patent family
member(s) | Publication
date |
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